bio-protocol Preprint

Not Peer Reviewed
Ask a question online

Allelic exchange constructs

EI Eva S Istvan AV Akhil Vaidya DG Daniel E Goldberg

Updated date: Jul 6, 2022

An abbreviated version of this protocol was published in eLIFE in Mar 2019

Plasmodium Niemann-Pick type C1-related protein is a druggable target required for parasite membrane homeostasis

DOI: 10.7554/eLife.40529

Detailed protocol

Protocol for preparation of allelic exchange parasites

Allelic exchange constructs were based on the pPM2GT vector (doi: 10.1083/jcb200307147). A C-terminal fragment of the ORF of PF3D7_0107500 was amplified using the primers ARF-F (CACTATAGAACTCGAGCCATCAAAAATTGTATCTATGGAAG) and ARF-R (CTGCACCTGGCCTAGGATGTAGTGGGCCAAAACTGGAAAGAAG). The pPM2GT vector was digested with XhoI and AvrII and gel purified. The PCR product was cloned into the digested pPM2GT vector using Infusion cloning (Takara Bio). The sequence of the construct was confirmed by Sanger sequencing. Using this strategy *pfncr1* is expressed from the endogenous promoter in-frame with a C-terminal GFP (the native stop is deleted). When desired, mutations were introduced into the *pfncr1* wild-type containing pPM2GT clone. Mutations typically contained changes to create compound-resistant parasites. Transfections were performed in duplicate using 100 mg of supercoiled DNA with 3D7 ring-stage parasites containing 10% parasites. To select for transfected parasites, 5 nM WR99210 was added to mature ring-stage parasites one cycle after transfection. Once transfectants grew, parasite cultures were cycled off for three weeks. Next WR99210 was reapplied until parasites grew well. The off-drug cycle was repeated for a second time to enrich for transfected parasites. Finally, transfectants were cloned by limited dilution.

Related files

Protocol for allelic exchange - eLife reader request.docx

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Istvan, E. S., Vaidya, A. and Goldberg, D. (2022). Allelic exchange constructs. Bio-protocol Preprint. bio-protocol.org/prep1772.
2. Istvan, E. S., Das, S., Bhatnagar, S., Beck, J. R., Owen, E., Llinas, M., Ganesan, S. M., Niles, J. C., Winzeler, E., Vaidya, A. B. and Goldberg, D. E.(2019). *Plasmodium* Niemann-Pick type C1-related protein is a druggable target required for parasite membrane homeostasis. eLIFE. DOI: 10.7554/eLife.40529

Copyright: Content may be subjected to copyright.